

We claim:

5 1. A method for simultaneous sequence-specific identification of mRNAs in a mRNA population comprising the steps of:

10 (a) preparing double-stranded cDNAs from a mRNA population using a mixture of 12 anchor primers, the anchor primers each including: (i) a tract of from 7 to 40 T residues; (ii) a site for cleavage by a restriction endonuclease that recognizes more than six bases, the site for cleavage being located to the 5'-side of the tract of T residues; (iii) a stuffer segment of from 4 to 40 nucleotides, the stuffer segment being located to the 5'-side of the site for cleavage by the restriction endonuclease; and (iv) phasing residues -V-N located at the 3' end of each of the anchor primers, wherein V is a deoxyribonucleotide selected from the group consisting of A, C, and G; and N is a deoxyribonucleotide selected from the group consisting of A, C, G, and T, the mixture including anchor primers containing all possibilities for V and N;

15 (b) producing cloned inserts from a suitable host cell that has been transformed by a vector, the vector having the cDNA sample that has been cleaved with a first restriction endonuclease and a second restriction endonuclease inserted therein, the cleaved cDNA sample being inserted in the vector in an orientation that is antisense with respect to a bacteriophage-specific promoter within the vector, the first restriction endonuclease recognizing a four-nucleotide sequence and the second restriction endonuclease cleaving at a single site within each member of the mixture of anchor primers;

20 (c) generating linearized fragments of the cloned inserts by digestion with at least one restriction endonuclease that is different from the first and second restriction endonucleases;

5 (d) generating a cRNA preparation of antisense
cRNA transcripts by incubation of the linearized
fragments with a bacteriophage-specific RNA polymerase
capable of initiating transcription from the
bacteriophage-specific promoter;

10 (e) dividing the cRNA preparation into sixteen
subpools and transcribing first-strand cDNA from each
subpool, using a thermostable reverse transcriptase and
one of sixteen primers whose 3'-terminus is -N-N, wherein
15 N is one of the four deoxyribonucleotides A, C, G, or T,
the primer being at least 15 nucleotides in length,
corresponding in sequence to the 3'-end of the
bacteriophage-specific promoter, and extending across
into at least the first two nucleotides of the cRNA, the
mixture including all possibilities for the 3'-terminal
two nucleotides;

20 (f) using the product of transcription in each
of the sixteen subpools as a template for a polymerase
chain reaction with a 3'-primer that corresponds in
sequence to a sequence in the vector adjoining the site
of insertion of the cDNA sample in the vector and a 5'-
primer selected from the group consisting of: (i) the
25 primer from which first-strand cDNA was made for that
subpool; (ii) the primer from which the first-strand cDNA
was made for that subpool extended at its 3'-terminus by
an additional residue -N, where N can be any of A, C, G,
or T; and (iii) the primer used for the synthesis of
first-strand cDNA for that subpool extended at its 3'-
terminus by two additional residues -N-N, wherein N can
30 be any of A, C, G, or T, to produce polymerase chain
reaction amplified fragments; and

 (g) resolving the polymerase chain reaction
amplified fragments by electrophoresis to display bands
representing the 3'-ends of mRNAs present in the sample.

2. The method of claim 1 wherein the anchor primers each have 18 T residues in the tract of T residues.

5 3. The method of claim 1 wherein the stuffer segment of the anchor primers is 14 residues in length.

10 4. The method of claim 3 wherein the sequence of the stuffer segment is A-A-C-T-G-G-A-A-G-A-A-T-T-C (SEQ ID NO: 1).

15 5. The method of claim 1 wherein the site for cleavage by a restriction endonuclease that recognizes more than six bases is the NotI cleavage site.

20 6. The method of claim 4 wherein the anchor primers have the sequence A-A-C-T-G-G-A-A-G-A-A-T-T-C-G-C-G-G-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N (SEQ ID NO: 2)

25 7. The method of claim 1 wherein the bacteriophage-specific promoter is selected from the group consisting of T3 promoter and T7 promoter.

30 8. The method of claim 7 wherein the bacteriophage-specific promoter is T3 promoter.

35 9. The method of claim 8 wherein the sixteen primers for priming of transcription of cDNA from cRNA have the sequence A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N-N (SEQ ID NO: 3).

10. The method of claim 1 wherein the vector is the plasmid pBC SK⁺ cleaved with ClaI and NotI and the 3'-primer in step (f) is G-A-A-C-A-A-A-G-C-T-G-G-A-G-C-T-C-C-A-C-C-G-C (SEQ ID NO: 4).

11. The method of claim 1 wherein the first restriction endonuclease recognizing a four-nucleotide sequence is MspI.

5 12. The method of claim 1 wherein the first restriction endonuclease recognizing a four-nucleotide sequence is selected from the group consisting of TagI and HinP1I.

10 13. The method of claim 1 wherein the restriction endonuclease cleaving at a single site in each of the mixture of anchor primers is NotI.

15 Sub 14. The method of claim 1 wherein the step of generating linearized fragments of the cloned inserts comprises:

Sub
By 16 (i) dividing the plasmid containing the insert into two fractions, a first fraction cleaved with the restriction endonuclease XhoI and a second fraction cleaved with the restriction endonuclease SalI;

20 (ii) recombining the first and second fractions after cleavage;

25 (iii) dividing the recombined fractions into thirds and cleaving the first third with the restriction endonuclease HindIII, the second third with the restriction endonuclease BamHI, and the third third with the restriction endonuclease EcoRI; and

30 (iv) recombining the thirds after digestion in order to produce a population of linearized fragments of which about one-sixth of the population corresponds to the product of cleavage by each of the possible combinations of enzymes.

35 15. The method of claim 1 wherein the mRNA population has been enriched for polyadenylated mRNA species.

16. The method of claim 1 wherein the intensity of each band displayed after electrophoresis is about proportional to the abundance of the mRNA corresponding to the band in the original mixture.

5

17. The method of claim 16 further comprising a step of determining the relative abundance of each mRNA in the original mixture from the intensity of the band corresponding to that mRNA after electrophoresis.

10

18. The method of claim 1 wherein the step of resolving the polymerase chain reaction amplified fragments by electrophoresis comprises electrophoresis of the fragments on at least two gels.

15

19. The method of claim 1 wherein the suitable host cell is Escherichia coli.

20

20. The method of claim 1 further comprising the steps of:

(h) eluting at least one cDNA corresponding to a mRNA from an electropherogram in which bands representing the 3'-ends of mRNAs present in the sample are displayed;

(i) amplifying the eluted cDNA in a polymerase chain reaction;

(j) cloning the amplified cDNA into a plasmid;

(k) producing DNA corresponding to the cloned DNA from the plasmid; and

(l) sequencing the cloned cDNA.

30

21. A method for simultaneous sequence-specific identification of mRNAs in a mRNA population comprising the steps of:

(a) isolating a mRNA population;

35

(b) preparing double-stranded cDNAs from the mRNA population using a mixture of 12 anchor primers with the sequence A-A-C-T-G-G-A-A-G-A-A-T-T-C-G-C-G-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-V-N (SEQ ID NO: 2), wherein V is a deoxyribonucleotide selected from the group consisting of A, C, and G; and N is a deoxyribonucleotide selected from the group consisting of A, C, G, and T, the mixture including anchor primers containing all possibilities for V and N, to produce a cDNA sample;

(c) cleaving the cDNA sample with two restriction endonucleases, a first restriction endonuclease MspI and a second restriction endonuclease NotI;

(d) inserting the cDNA sample cleaved with the first and second restriction endonucleases into a vector, the cleaved cDNA being inserted in an orientation that is antisense with respect to a T3 promoter within the vector, the vector being the plasmid pBC SK⁺ cleaved with *Cla*I and *Not*I;

(e) transforming Escherichia coli with the vector into which the cleaved cDNA has been inserted to produce cloned inserts;

(f) generating linearized fragments of the cloned inserts by digestion with at least one restriction endonuclease that is different from the first and second restriction endonucleases;

(g) generating a cRNA preparation of antisense cRNA transcripts by incubation of the linearized fragments with a T3 RNA polymerase capable of initiating transcription from the T3-specific promoter;

(h) dividing the cRNA preparation into sixteen subpools and transcribing first-strand cDNA from each subpool, using a thermostable reverse transcriptase, and one of the sixteen primers A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N-N (SEQ ID NO: 3), wherein N is one of the four

deoxyribonucleotides A, C, G, or T, the mixture including all possibilities for the 3'-terminal two nucleotides;

5 (i) using the product of transcription in each of the sixteen subpools as a template for a polymerase chain reaction with the 3'-primer G-A-A-C-A-A-A-G-C-T-G-G-A-G-C-T-C-C-A-C-C-G-C (SEQ ID NO: 4), and a 5'-primer selected from the group consisting of: (1) the primer from which first-strand cDNA was made for that subpool; (2) the primer from which the first-strand cDNA was made for that subpool extended at its 3'-terminus by an additional residue -N, where N can be any of A, C, G, or T; and (3) the primer used for the synthesis of first-strand cDNA for that subpool extended at its 3'-terminus by two additional residues -N-N, wherein N can be any of A, C, G, or T, to produce polymerase chain reaction amplified fragments; and

10 (j) resolving the polymerase chain reaction amplified fragments by electrophoresis to display bands representing the 3'-ends of mRNAs present in the sample.

20 22. A method of simultaneous sequence-specific identification of mRNAs corresponding to members of an antisense cRNA pool representing the 3'-ends of a population of mRNAs, the antisense cRNAs that are members of the antisense cRNA pool being terminated at their 5'-end with a primer sequence corresponding to a bacteriophage-specific vector and at their 3'-end with a sequence corresponding in sequence to a sequence of the vector, the method comprising:

25 (a) dividing the members of the antisense cRNA pool into sixteen subpools and transcribing first-strand cDNA from each subpool, using a thermostable reverse transcriptase and one of sixteen primers whose 3'-terminus is -N-N, wherein N is one of the four deoxyribonucleotides A, C, G, or T, the primer being at least 15 nucleotides in length, corresponding in sequence

to the 3'-end of the bacteriophage-specific promoter, and extending across into at least the first two nucleotides of the crRNA, the mixture including all possibilities for the 3'-terminal two nucleotides;

23. A method for detecting a change in the pattern of mRNA expression in a tissue associated with a physiological or pathological change comprising the steps of:

5 (c) obtaining a second sample of the tissue
that has been subject to the physiological or
pathological change;

10 (d) determining the pattern of mRNA expression
in the second sample of the tissue by performing steps
(a)-(c) of claim 22 to generate a second display of bands
representing the 3'-ends of mRNAs present in the second
sample; and

15 (e) comparing the first and second displays to
determine the effect of the physiological or pathological
change on the pattern of mRNA expression in the tissue.

20 24. The method of claim 23 wherein the tissue
is derived from the central nervous system.

25 25. The method of claim 24 wherein the
physiological or pathological change is selected from the
group consisting of Alzheimer's disease, parkinsonism,
ischemia, alcohol addiction, drug addiction,
schizophrenia, amyotrophic lateral sclerosis, multiple
sclerosis, depression, and bipolar manic-depressive
disorder.

30 26. The method of claim 24 wherein the
physiological or pathological change is associated with
learning or memory, emotion, glutamate neurotoxicity,
feeding behavior, olfaction, vision, movement disorders,
viral infection, electroshock therapy, or the
administration of a drug or toxin.

35 27. The method of claim 24 wherein the
physiological or pathological change is selected from the
group consisting of circadian variation, aging, and long-
term potentiation.

5 28. The method of claim 24 wherein the tissue
is derived from a structure within the central nervous
system selected from the group consisting of retina,
cerebral cortex, olfactory bulb, thalamus, hypothalamus,
anterior pituitary, posterior pituitary, hippocampus,
nucleus accumbens, amygdala, striatum, cerebellum, brain
stem, suprachiasmatic nucleus, and spinal cord.

10 29. The method of claim 23 wherein the tissue
is from an organ or organ system selected from the group
consisting of the cardiovascular system, the pulmonary
system, the digestive system, the peripheral nervous
system, the liver, the kidney, skeletal muscle, and the
reproductive system.

15 30. A method of screening for a side effect of
a drug comprising the steps of:

20 (a) obtaining a first sample of tissue from an
organism treated with a compound of known physiological
function;

25 (b) determining the pattern of mRNA expression
in the first sample of the tissue by performing steps
(a)-(c) of claim 22 to generate a first display of bands
representing the 3'-ends of mRNAs present in the first
sample;

30 (c) obtaining a second sample of tissue from
an organism treated with a drug to be screened for a side
effect;

35 (d) determining the pattern of mRNA expression
in the second sample of the tissue by performing steps
(a)-(c) of claim 22 to generate a second display of bands
representing the 3'-ends of mRNAs present in the second
sample; and

40 (e) comparing the first and second displays in
order to detect the presence of mRNA species whose
expression is not affected by the known compound but is

affected by the drug to be screened, thereby indicating a difference in action of the drug to be screened and the known compound and thus a side effect.

5 31. The method of claim 30 wherein the drug to be tested is selected from the group consisting of antidepressants, neuroleptics, tranquilizers, anticonvulsants, monoamine oxidase inhibitors, and stimulants.

10 32. The method of claim 30 wherein the drug to be tested is selected from the group consisting of anti-parkinsonism agents, skeletal muscle relaxants, analgesics, local anesthetics, cholinergics, antiviral agents, antispasmodics, steroids, and non-steroidal anti-inflammatory drugs.

15 33. A panel of primers comprising 16 primers of the sequence A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N-N (SEQ ID NO: 3), wherein N is one of the four deoxyribonucleotides A, C, G, or T.

20 34. A panel of primers comprising 64 primers of the sequences A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N-N-N (SEQ ID NO: 5), wherein N is one of the four deoxyribonucleotides A, C, G, or T.

25 35. A panel of primers comprising 256 primers of the sequences A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N-N-N-N (SEQ ID NO: 6), wherein N is one of the four deoxyribonucleotides A, C, G, or T.

30 36. A panel of primers comprising 12 primers of the sequences A-A-C-T-G-G-A-A-G-A-A-T-T-C-G-C-G-G-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-V-N (SEQ ID NO: 2), wherein V is a deoxyribonucleotide

selected from the group consisting of A, C, and G; and N is a deoxyribonucleotide selected from the group consisting of A, C, G, and T.

5 37. A degenerate mixture of primers comprising a mixture of 12 primers of the sequences A-A-C-T-G-G-A-A-G-A-A-T-T-C-G-C-G-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N (SEQ ID NO: 2), wherein V is a deoxyribonucleotide selected from the group consisting of A, C, and G; and N is a deoxyribonucleotide selected from the group consisting of A, C, G, and T, each of the 12 primers being present in about an equimolar quantity.

10

00064557-052802